

## Increases in insulin-like growth factor-1 level and peroxidative damage after gestational ethanol exposure in rats<sup>☆</sup>

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### Abstract

Ethanol exposure during pregnancy elicits profound detrimental developmental and behavioral effects such as reduced levels of insulin-like growth factor-1 (IGF-1) in the fetus. However, few reports have addressed its impact on postpartum dams. This study was designed to examine the influence of gestational ethanol exposure on postpartum maternal organ oxidative damage and IGF-1 level. Pregnant female rats were pair-fed from Day 2 of gestation until labor with control or ethanol (6.36% (v/v)) liquid diets and were sacrificed 6 weeks after parturition (ethanol withdrawn after parturition). There was no difference in body weight during or after the gestational period between the control and ethanol groups. Litter size was significantly less for ethanol-fed dams. One-week postnatal pup survival was significantly lower in the ethanol-fed (57.1%) than the control (97.8%) group. Liver and kidney tissue IGF-1 levels and mRNA were elevated in the ethanol-fed mothers, accompanied by hepatic but not renal oxidative damage, indicated by profound lipid peroxidation (measured by malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE)) and protein carbonyl formation. The levels of glutathione (GSH), glutathione disulfide (GSSG) and GSH/GSSG ratios in liver and kidney were not different between the ethanol-fed and control dams. Collectively, these data suggest that gestational ethanol exposure may lead to postpartum oxidative organ damage and a possible compensatory increase in organ IGF-1 levels.

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### 1. Introduction

The fetotoxic effects of maternal ethanol exposure have been documented for decades, yet the mechanisms underlying this devastating phenomenon remain unclear. The wide variety of cellular and biochemical effects of ethanol on not only the fetal but also on the maternal organs is itself a puzzle and implies that ethanol toxicity may be multifac-

torial. Several hypotheses have been proposed concerning the toxic effects of ethanol, including its effects on membrane structure and function, as well as protein synthesis [1,2]. Recently, compelling evidence suggests a link between ethanol intake and enhanced oxidative injury [3,4]. Short-term maternal ethanol exposure has been shown to induce lipid peroxidation represented as enhanced hepatic levels of 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) in both the adult and the fetus [5,6]. Both 4-HNE and MDA may inhibit cytochrome *c* oxidase by forming adducts with the enzyme subunits, and therefore may be responsible for enzymatic inhibition by ethanol [6]. It has been shown that elevated MDA and 4-HNE may contribute directly to oxidative stress and/or injury [7].

Ethanol exposure during pregnancy has profound detrimental consequences on maternal organs. The levels of maternal circulating insulin-like growth factor-1 (IGF-1) and hepatic IGF-1 mRNA were both significantly reduced by ethanol exposure during pregnancy [8]. This is consistent

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with the notion that chronic, heavy ethanol ingestion inhibits plasma IGF-1 levels and gene expression, resulting in a long-term reduction in circulating and tissue IGF-1 and its binding proteins IGFBPs [9]. However, light to moderate intake of ethanol has been shown to result in an acute increase in IGFBP-1 and IGF-1. The mechanism behind the IGFBP-1 increase may be due to a direct hepatic effect [9]. Nevertheless, these data indicate that ethanol exposure is associated with changes in IGF-1 regulation, and contributes to maternal and/or placental metabolic changes during pregnancy, therefore affecting postnatal growth [8]. IGF-1 is a mitogenic peptide synthesized mainly (~90%) in the liver. IGF-1 plays an important role in DNA and RNA synthesis and developmental growth/maturation [10]. Because IGF-1 may have a significant role in maternal and placental metabolism, hormonal regulation and oxidative stress defense [11] the objective of the present investigation was to study the impact of gestational ethanol exposure on postpartum organ IGF-1 levels and oxidative stress/injury.

## 2. Materials and methods

### 2.1. Gestational ethanol exposure animal model

The experimental protocol used in this study was approved by our Institutional Animal Care and Use Committee at University of North Dakota (Grand Forks, ND, USA). Adult nulliparous female Sprague–Dawley rats (~190 g) were obtained from an in-house colony and were mated with male rats overnight. The presence of a vaginal plug the following morning indicated successful mating and was designated gestational day 1 (GD1). The pregnant rats were randomly divided into two groups and were introduced to a liquid diet (Shake & Pour BioServ Inc., Frenchtown, NJ) with or without ethanol supplementation, beginning on GD2 until the pups were born (usually around GD21). This liquid diet has been used extensively in our laboratory and proven to be nutritionally complete [12]. The ethanol-containing diet had 36% of the total calories isocalorically replaced by ethanol. As with most chronic ethanol studies, an isocaloric pair-feeding regimen was employed to eliminate the possibility of nutritional deficits [12]. In this instance, non-ethanol-consuming animals were offered the same quantity of diet ethanol-fed animals drank the previous day. The amount of diet consumed and animal body weights were measured daily and weekly, respectively. All animals were housed in a temperature-controlled room under a 12/12-h light/dark illumination cycle. Maternal blood samples were collected on GD20. Plasma ethanol concentrations of the dams were determined using the alcohol dehydrogenase assay with samples collected at random both during the feeding regime and after sacrifice (Sigma Chemical, St. Louis, MO). Both ethanol-fed and control dams were maintained for another 6 weeks on non-ethanol-containing regular rat chow ad libitum after parturition before being sacrificed for

study. Litter size, birth weights and postnatal pup survival were determined for each dam.

### 2.2. Measurement of organ IGF-1 levels

Excised liver and kidneys from the 6-week postpartum dams were immediately frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$  until lyophilization. Levels of IGF-1 in these organs were determined using a modified procedure described previously [13]. Briefly, the lyophilized organs were pulverized and duplicate aliquots of each organ were extracted with 1 M acetic acid by end-to-end rotation for 4 h at  $4^{\circ}\text{C}$ . The extraction mixtures were centrifuged. The IGF-1 concentration of the supernatant was determined using a competitive binding enzyme immunoassay kit (Diagnostic Systems Laboratories Inc., Webster, TX) after pretreatment to remove IGF-1 binding proteins. The recovery of an internal standard of IGF-1 added at the beginning of the extraction procedure was approximately 99% for both tissues. The inter- and intra-assay variability is 4.9–11.9% and 5.3–9.1%, respectively.

### 2.3. Measurement of organ IGF-1 mRNA

To quantitate IGF-1 mRNA levels, total RNA was extracted from livers or kidneys using the guanidine thiocyanate method [14]. The RNA was visualized using ethidium bromide staining to determine RNA integrity and the equal loading of lanes. Differences in loading were normalized using the fluorescence intensity of the 18S band as measured by the gel documentation system (LumiAnalyst, Boehringer Mannheim, Indianapolis, IN). The RNA was transferred to nylon membranes, dried, and hybridized (overnight at  $65^{\circ}\text{C}$ ) with a 376 bp,  $^{32}\text{P}$ -labeled antisense riboprobe generated from an IGF-1 cDNA (pGEM3 vector) kindly provided by Dr. Derek LeRoith at National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, MD). Membranes were washed at  $68^{\circ}\text{C}$  and were exposed to film to visualize IGF-1 mRNA levels. Band intensities were compared using densitometric measurement to determine relative IGF-1 mRNA levels.

### 2.4. Measurement of glutathione and glutathione disulfide (GSH and GSSG)

For measurement of GSH, frozen tissue samples were homogenized in four volumes (w/v) of 1% picric acid. Acid homogenates were centrifuged at  $16,000 \times g$  (30 min) and supernatant fractions collected. Supernatant fractions were assayed for total GSH and GSSG by the standard recycling method [15] and GSH content determined using a standard curve generated from known concentrations of GSH. The procedure consisted of using one-half of each sample for GSSG determination and the other half for GSH. Samples for GSSG determination were incubated at room temperature with  $2\text{ }\mu\text{l}$  of 4-vinyl pyridine (4-VP) per  $100\text{ }\mu\text{l}$  sample for 1 h after vigorous vortexing. Incubation with 4-VP

conjugates any GSH present in the sample so that only GSSG is recycled to GSH in the recycling assay. This allowed for measurement of only GSSG without interference by GSH. The GSSG (as GSHx2) was then subtracted from the total glutathione to determine actual GSH level [16].

### 2.5. Protein carbonyl assay

To assess oxidative protein damage in liver and kidney tissues, the carbonyl content of protein extracted from tissues was determined using the published method [17]. Since drastic homogenization may result in breakage of nuclei and release of nucleic acid, approximately 200 mg wet tissues were placed in small plastic dishes containing lysis buffer and finely minced before being incubated for 15 min at room temperature. Nucleic acids were eliminated by treating the samples with 1% streptomycin sulfate for 15 min, followed by a 10 min centrifugation ( $11,000 \times g$ ). Protein was precipitated by adding an equal volume of 20% TCA to protein (0.5 mg) and centrifuged for 1 min. The TCA solution was removed and the sample resuspended in 10 mM 2,4-dinitrophenylhydrazine (2,4-DNPH) solution. Samples were incubated at room temperature for 15–30 min. Five hundred microliter of 20% TCA was then added and samples were centrifuged for 3 min. The supernatant was discarded, the pellet washed in ethanol:ethyl acetate and allowed to incubate at room temperature for 10 min. The samples were centrifuged again for 3 min and the ethanol:ethyl acetate steps repeated two more times. The precipitate was resuspended in 6 M guanidine solution, centrifuged for 3 min and insoluble debris removed. The maximum absorbance (360–390 nm) of the supernatant was read against appropriate blanks (water, 2 M HCl) and the carbonyl content was calculated using the molar absorption coefficient of  $22,000 \text{ M}^{-1} \text{ cm}^{-1}$ .

### 2.6. Lipid peroxidation assay

To assess lipid peroxidation, MDA and 4-HNE levels were measured in liver and kidney homogenates. A colorimetric assay (LPO-586 Kit; Oxis International, Portland, OR) based on the method of [18] was used to determine levels of lipid peroxidation. Briefly, a 20–30% tissue homogenate was prepared using 20 mM Tris buffer containing 5 mM butylated hydroxytoluene (BHT) to prevent sample oxidation. The protein concentration was determined using the Bradford method. Protein samples were stored at  $-70^\circ\text{C}$  until analysis. To 200  $\mu\text{l}$  of sample, 10  $\mu\text{l}$  of 0.5 M BHT, and 650  $\mu\text{l}$  of Reagent 1 were added. The tubes were gently vortexed and 150  $\mu\text{l}$  of 15.4 M methanesulfonic acid (Reagent 2) were added. Samples were incubated at  $45^\circ\text{C}$  for 45 min followed by centrifugation for 10 min at  $15,000 \times g$ . The clear supernatant was transferred to a cuvette and the absorbance measured at 586 nm. Sample blanks, reagent blanks and MDA and 4-HNE standards were included each time the assay was performed. The concentration of the analyte

in each sample was calculated using the molar extinction coefficient at 586 nm of 110,000.

### 2.7. Statistical analyses

For each experimental series, data are presented as mean  $\pm$  S.E.M. Statistical significance ( $P < 0.05$ ) for each variable was estimated by Student's *t*-test.

## 3. Results

### 3.1. General features of experimental animals and fetal outcome

The average daily intake of the ethanol liquid diet was  $51.3 \pm 0.2 \text{ ml}$ . The control group was pair-fed, receiving the same amount of diet their ethanol counterparts consumed the previous day. The effects of gestational ethanol consumption on gestational and postgestational growth are shown in Fig. 1. Gestational ethanol intake did not affect body growth either during or after the gestation. Table 1 exhibits the body, heart, liver and kidney weights at the time of sacrifice as well as ethanol concentrations on GD20. Ethanol-fed dams showed slightly larger body weights (although not statistically significant), but smaller heart, liver and kidney sizes (organ-to-body weight ratio), compared to control animals. The absolute heart, liver and kidney weights were not different between the two groups (data not shown for liver and kidney). As expected, plasma ethanol levels (obtained on GD20) were greater among ethanol-consuming dams compared to the controls. The average birth weight (measured on Day 2) of all litters was significantly ( $P < 0.05$ ) lower in the gestational ethanol-fed group ( $4.87 \pm 0.16 \text{ g}$ ,  $n = 7$  litters with a total of 56 pups) compared to control ( $6.09 \pm 0.39 \text{ g}$ ,  $n = 8$  litters with a total of 46 pups). In addition, the 1-week postnatal survival for the pups was decreased from 97.8% in controls to 57.1% in ethanol-fed group, indicating potential teratogenic effects of ethanol.

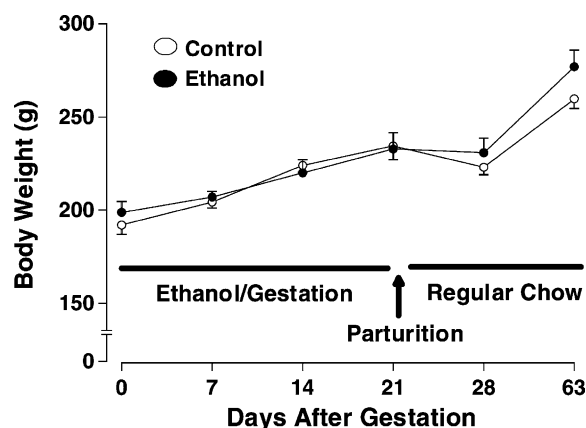


Fig. 1. Body weight of female rats during gestational and postpartum periods. Gestational ethanol exposure was applied to the ethanol group with pair-fed control. Mean  $\pm$  S.E.M.,  $n = 8$  rats per group.

Table 1  
General features of dams consuming control or ethanol diets

Rat group (n)	Body weight (g)	Heart weight (g)	Heart weight/body weight (mg g <sup>-1</sup> )	Liver weight/body weight (mg g <sup>-1</sup> )	Kidney weight/body weight (mg g <sup>-1</sup> )	Plasma alcohol (mg dl <sup>-1</sup> )
Control (8)	260 ± 5	1.39 ± 0.06	5.34 ± 0.15	35.7 ± 0.5	6.96 ± 0.12	0.00 ± 0.00
Ethanol (8)	277 ± 9	1.26 ± 0.08	4.55 ± 0.22*	32.1 ± 1.0*	6.51 ± 0.17*	17.62 ± 4.09*

Mean ± S.E.M., \**P* < 0.05 vs. control group, (n): number of animals.

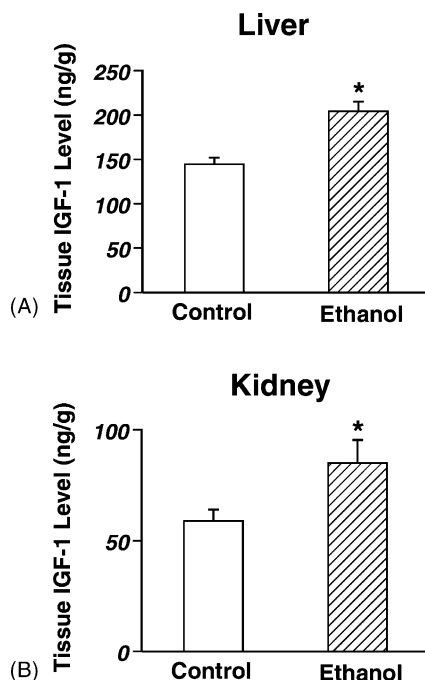


Fig. 2. Organ IGF-1 levels in livers (A) and kidneys (B) from postpartum rats with gestational ethanol exposure and pair-fed control. Mean ± S.E.M., *n* = 8, \**P* < 0.05 vs. control.

### 3.2. Effect of gestational ethanol intake on tissue IGF-1 and IGF-1 mRNA levels

Gestational ethanol intake caused a significant increase in IGF-1 levels in both liver (ethanol: 204 ± 11 versus control: 144 ± 7 ng g<sup>-1</sup> dry weight, *P* < 0.05) and kidney (ethanol: 85 ± 11 versus control: 59 ± 5 ng g<sup>-1</sup>, *P* < 0.001) compared with the control group (Fig. 2). The liver concentration of IGF-1 was approximately two-fold of that found in the kidneys in either control or ethanol group. The gestational ethanol exposure-induced elevation in organ IGF-1 levels was supported by an enhanced liver IGF-1 mRNA level, although to a lesser extent (Fig. 3).

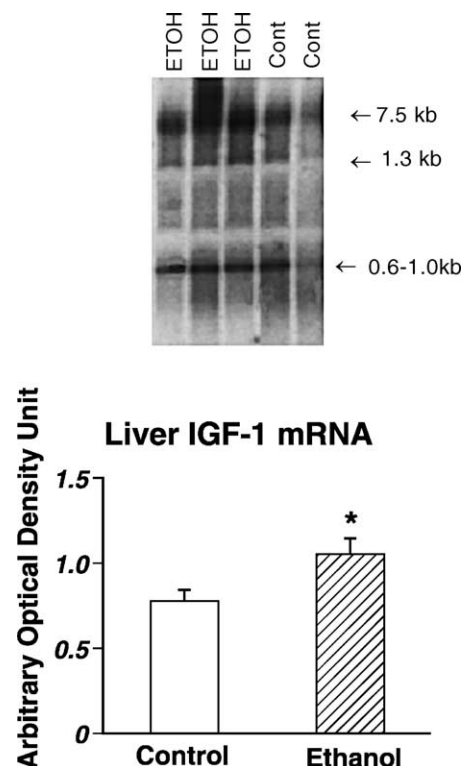


Fig. 3. Liver IGF-1 mRNA levels in postpartum rats with gestational ethanol exposure and pair-fed control. Inset shows a representative Northern gel blot. The 7.5 kb band was used for quantification purpose. Mean ± S.E.M., *n* = 3, \**P* < 0.05 vs. control.

### 3.3. Effect of gestational ethanol intake on GSH/GSSG levels, lipid peroxidation and protein carbonyl formation in postpartum mothers

The GSH, GSSG levels are commonly used markers for oxidative stress. A low GSH/GSSG ratio suggests increased oxidative stress. Results in Table 2 indicate that gestational ethanol exposure did not significantly affect GSH, GSSG levels or their ratio in either organ. The liver

Table 2  
Levels of glutathione (GSH) and glutathione disulfide (GSSG) in postpartum dams with or without gestational ethanol exposure

Group (n)	Glutathione (GSH, nmol mg <sup>-1</sup> tissue)	Glutathione disulfide (GSSG, nmol mg <sup>-1</sup> tissue)	GSH/GSSG
Liver-control (8)	333.4 ± 12.1	162.5 ± 0.7	2.05 ± 0.07
Liver-ethanol (8)	328.6 ± 9.0	160.5 ± 0.5	2.05 ± 0.06
Kidney-control (8)	16.45 ± 0.25	8.53 ± 0.15	1.93 ± 0.01
Kidney-ethanol (8)	16.45 ± 0.19	8.38 ± 0.11	1.96 ± 0.03

Mean ± S.E.M., (n): number of animals.

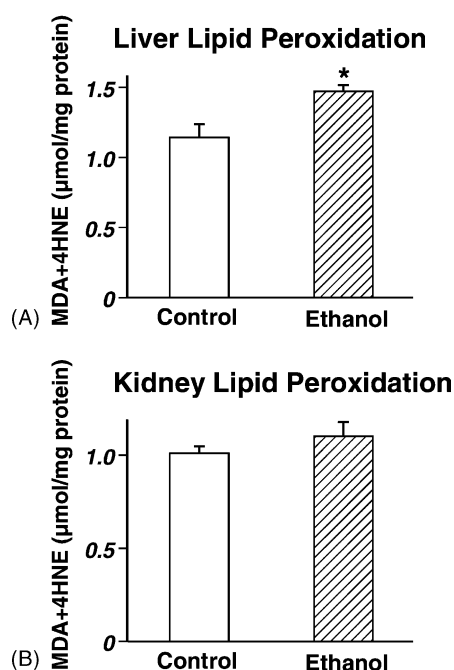


Fig. 4. Lipid peroxidation levels (malondialdehyde and 4-hydroxynonenal) in livers and kidneys from postpartum rats with gestational ethanol exposure and pair-fed control. Mean  $\pm$  S.E.M.,  $n = 8$ , \* $P < 0.05$  vs. control.

lipid peroxidation represented as MDA + 4-HNE levels was significantly elevated in the gestational ethanol-fed group (Fig. 4). However, lipid peroxidation was not altered in the kidney. Consistent with the lipid peroxidation data, protein carbonyl formation was significantly enhanced in liver but not kidney from the gestational ethanol-fed group compared to the control group (Fig. 5).

#### 4. Discussion

The major finding of this study is that IGF-1 and IGF-1 mRNA levels in livers and kidneys are elevated in postpartum dams after gestational ethanol exposure. Also, lipid peroxidation and protein carbonyl contents were increased in the gestational ethanol exposure group. IGF-1 has been shown to be an antioxidant and an indicator for oxidative stress [19]. The increased IGF-1 levels may be compensatory to the elevated hepatic lipid peroxidation and protein carbonyl levels in response to gestational ethanol exposure. It appears that extrahepatic tissues such as the kidney may not be as sensitive to ethanol-induced oxidative damage. These data indicate an overall elevated oxidative tissue damage in postpartum mothers following gestational ethanol intake.

The nutritional status of the experimental animals is an important variable that may influence the IGF-1 level and tissue oxidative stress. In this study, the control group was pair-fed with the ethanol group using isocaloric-nutritionally complete liquid diets (Shake & Pour BioServ Inc.). The

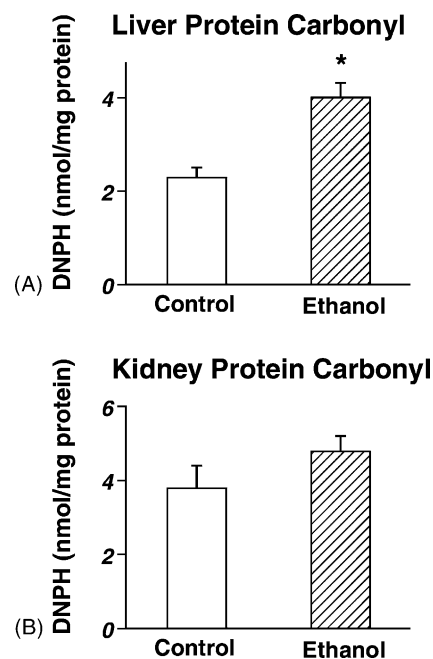


Fig. 5. Protein carbonyl formation in livers and kidneys from postpartum rats with gestational ethanol exposure and pair-fed control. Mean  $\pm$  S.E.M.,  $n = 8$ , \* $P < 0.05$  vs. control.

use of the liquid diet is based on the observations that ethanol self-administration resulted in less nutritional deficiencies and less stress to the animals in comparison to forced-feeding regimens, intravenous administration, or aerosolized inhalation [20]. The blood ethanol level detected in our experimental animals ( $\sim 18 \text{ mg dl}^{-1}$ ) is relatively low, considering the fact that longer feeding (12 weeks) using the same liquid diet may attain peak blood ethanol levels of over  $200 \text{ mg dl}^{-1}$  [12], and also the fact that  $90 \text{ mg dl}^{-1}$  as a moderate ethanol intake dose (national legal driving limit is  $100 \text{ mg dl}^{-1}$ ). Therefore, our study may reflect a light to moderate drinking over the gestational period. However, pattern drinking (e.g. binge drinking) and the fact that pregnant rats do not voluntarily consume ethanol may also play a role in the peak blood ethanol level [21]. Our findings indicate a slightly elevated (although not significant) body weight in the ethanol exposure group compared to the control group, which may be mainly responsible for the reduced organ size (proportional to body weight). One of the more important findings from our study is the increased tissue MDA and 4-HNE levels following gestational ethanol exposure, even with 6 weeks postpartum ethanol abstinence. These data suggest that gestational ethanol exposure may lead to irreversible damage in both lipids and proteins. However, it appears that hepatic tissues have a higher susceptibility to ethanol-induced damage compared with extrahepatic tissues such as the kidney. This may reflect a higher susceptibility to MDA and 4-HNE formation in addition to a reduced capacity for their metabolism, which may directly contribute to oxidative damage supported by hepatic protein carbonyl formation.



Oxidative stress occurs due to a disturbance in the pro-oxidant and antioxidant molecules or enzymes whereby resulting in a pro-oxidant predominant environment. This has been established to be a pertinent factor in chronic ethanol intake as well as in other pathological conditions, such as diabetes and ovarian cancer [3,22]. Oxidative stress is mainly mediated by free radical generation, resulting in damage to proteins and lipids and allowing calcium ion accumulation, consequently leading to cell death [23]. Interestingly, the levels of GSH and GSSH, which serve as important indicators of the redox environment [24], were not altered 6-week postparturition during which time ethanol was withdrawn from the diet. These negative findings seem to indicate that the oxidative stress status no longer exists once ethanol is removed after short-term exposure, although permanent irreversible damage has occurred. Conflicting results on the hepatic content of GSH have been observed in experimental animals after chronic ethanol administration. While some investigators reported no change in GSH content, others found that it either decreased or increased [25]. The discrepancies may be ascribed to differences in the experimental designs and procedures of GSH measurement. Nevertheless, other than the most abundant redox couple GSH/GSSG, there are other auto-oxidative mechanisms to maintain the cellular redox environment, which warrant further study.

Chronic ethanol intake alters the levels of IGF-1 and their serum-binding proteins, leading to alterations in organ function and morphology [9]. Results from the present study show that IGF-1 levels are significantly elevated in liver and kidney following short-term gestational ethanol intake. These findings support the view that tissue anti-oxidant status (such as IGF-1) may be a significant element in the etiology of alcoholism [3]. IGF-1 has been shown to protect against oxidative stress and apoptosis [26]. However, the mechanism(s) of action behind the enhanced IGF-1 levels is not fully understood. IGF-1 is a potent anabolic agent that plays an important role in regulating muscle protein balance. Alterations in one or more of the various components of oxidative stress pathways may be responsible for the enhanced compensatory increase in tissue IGF-1 levels in postpartum tissues. It is worth mentioning that our findings are contrary to the findings of reduced IGF-1 levels immediately after parturition [8]. One plausible explanation may be that 6 weeks of an ethanol-free period may have given the body the time needed to “respond” to the enhanced oxidative damage. However, long-term (8 weeks) chronic ethanol intake leads to a decreased plasma as well as hepatic IGF-1 level compared with pair-fed control animals [9,27,28]. IGF-1 content in extrahepatic tissues such as the duodenum and kidney, however, was not altered by ethanol exposure [9]. Concomitantly, the relative concentration of IGF binding protein IGFBP-1 was increased in plasma, liver, and muscle of ethanol-fed rats. These data indicate that chronic ethanol feeding decreases IGF-1 and increases IGFBP-1 in the circulation and in skeletal muscle and that these changes

appear to be independent of changes in classical hormonal regulators of the IGF system. The observed alterations in the IGF system are consistent with a reduction in the anabolic actions of IGF-1 induced by chronic ethanol intake [29].

As mentioned, IGF-1 is not only a growth factor but also a potent antioxidant. Antioxidants prevent new radical species formation by converting existing free radical species into less harmful molecules, or by preventing transformation of free radicals from other molecules. Elevated levels of an antioxidant system(s) often develop in response to increased oxidative stress including chronic alcoholism, in order to assist the tissues under oxidative attack to build adequate defense against reactive oxygen species. In conclusion, our study provides helpful information towards understanding the cellular mechanisms associated with gestational ethanol exposure and subsequently, postpartum oxidative stress.

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### References

- [1] Preedy VR, Richardson PJ. Ethanol-induced cardiovascular disease. *Br Med Bull* 1994;50:152–63.
- [2] Baraona E, Lieber CS. Alcohol and lipids. *Recent Dev Alcohol* 1998;14:97–134.
- [3] McDonough KH. The role of alcohol in the oxidant antioxidant balance in heart. *Front Biosci* 1999;4:d601–6.
- [4] Niemela O. Distribution of ethanol-induced protein adducts in vivo: relationship to tissue injury. *Free Radic Biol Med* 2001;31:1533–8.
- [5] Chen JJ, Schenker S, Henderson GI. 4-Hydroxynonenal levels are enhanced in fetal liver mitochondria by in utero ethanol exposure. *Hepatology* 1997;25:142–7.
- [6] Chen J, Petersen DR, Schenker S, Henderson GI. Formation of malondialdehyde adducts in livers of rats exposed to ethanol: role in ethanol-mediated inhibition of cytochrome *c* oxidase. *Alcohol Clin Exp Res* 2001;24:544–52.
- [7] Meagher EA, Fitzgerald GA. Indices of lipid peroxidation in vivo: strengths and limitations. *Free Radic Biol Med* 2000;28:1745–50.
- [8] Breese CR, Sonntag WE. Effect of ethanol on plasma and hepatic insulin-like growth factor regulation in pregnant rats. *Alcohol Clin Exp Res* 1995;19:867–73.
- [9] Sonntag WE, Boyd RL. Diminished insulin-like growth factor-1 levels after chronic ethanol: relationship to pulsatile growth hormone release. *Alcohol Clin Exp Res* 1989;13:3–7.
- [10] D’Ercole AJ. The insulin-like growth factors and fetal growth. In: Spencer M, editor. *Modern concepts of insulin-like growth factors*. New York: Elsevier; 1991. p. 9–24.
- [11] Breese CR, Ingram RL, Sonntag WE. Influence of age and long-term dietary restriction on plasma insulin-like growth factor-1 (IGF-1), IGF-1 gene expression, and IGF-1 binding proteins. *J Gerontol* 1991;46:B180–7.

- [12] Ren J, Brown RA. Influence of chronic alcohol ingestion on acetaldehyde-induced depression of cardiac contractile function. *Alcohol Alcoholism* 2000;35:554–60.
- [13] D'Ercole AJ, Stiles AD, Underwood LE. Tissue concentrations of somatomedin C: further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of action. *Proc Natl Acad Sci USA* 1984;81:935–59.
- [14] Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate phenol-chloroform extraction. *Ann Biochem* 1987;162:156–9.
- [15] Griffith OW. Glutathione and glutathione disulfide. In: Bergmeyer HU, Bergmeyer J, editors. *Methods of enzymatic analysis*. Deerfield Beach, FL; 1986. p. 521–9.
- [16] Brown-Borg HM, Bode AM, Bartke A. Antioxidative mechanisms and plasma growth hormone levels: potential relationship in the aging process. *Endocrine* 1999;11:41–8.
- [17] Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, et al. Determination of carbonyl content in oxidatively modified proteins. *Method Enzyme* 1990;186:464–78.
- [18] Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radic Biol Med* 1991;11:81–128.
- [19] Ren J, Samson WK, Sowers JR. Insulin-like growth factor-1 as a cardiac hormone: physiological and pathophysiological implications in heart disease. *J Mol Cell Cardiol* 1999;31:2049–61.
- [20] Keane B, Leonard BE. Rodent models of alcoholism: a review. *Alcohol Alcoholism* 1989;24:299–309.
- [21] Zajac CS, Albel EL. Animal models of prenatal alcohol exposure. *Int J Epidemiol* 1992;21:S24–32.
- [22] Henderson GI, Chen JJ, Schenker S. Ethanol, oxidative stress, reactive aldehydes, and the fetus. *Front Biosci* 1999;4:D541–50.
- [23] Wattanapitayakul SK, Bauer JA. Oxidative pathways in cardiovascular disease: roles, mechanisms, and therapeutic implications. *Pharmacol Ther* 2001;89:187–206.
- [24] Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic Biol Med* 2001;30:1191–212.
- [25] Fernandez-Checa JC, Hirano T, Tsukamoto H, Kaplowitz N. Mitochondrial glutathione depletion in alcoholic liver disease. *Alcohol* 1993;10:469–75.
- [26] Parrizas M, LeRoith D. Insulin-like growth factor-1 inhibition of apoptosis is associated with increased expression of the bcl-xL gene product. *Endocrinology* 1997;138:1355–8.
- [27] Sonntag WE, Boyd RL. Chronic ethanol feeding inhibits plasma levels of insulin-like growth factor-1. *Life Sci* 1998;43:1325–30.
- [28] Rojdmarm S, Rydvald Y, Aquilonius A, Brismar K. Insulin-like growth factor (IGF)-1 and IGF-binding protein-1 concentrations in serum of normal subjects after alcohol ingestion: evidence for decreased IGF-1 bioavailability. *Clin Endocrinol* 2000;52:313–8.
- [29] Lang CH, Fan J, Lipton BP, Potter BJ, McDonough KH. Modulation of the insulin-like growth factor system by chronic alcohol feeding. *Alcohol Clin Exp Res* 1998;22:823–9.